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Diamond-like Carbon Coating on Micropipettes

N. Kakuta[1], N. Okuyama[2], M. Watanabe[1], Y. Imai[1], K. Mabuchi[3], and Y. Yamada[1]

[1] Dept. Mechanical Engineering and Intelligent Systems, The University of Electro-Communications, Chofu, Japan
[2] Dept. Electronic Engineering, The University of Electro-Communications, Chofu, Japan
[3] Graduate School of Information Science and Technology, The University of Tokyo, Tokyo, Japan

Abstract—**Based on glass micropipettes which are widely used in bioengineering and medicine, various micro-sensor probes could be produced by recent microfabrication techniques. Conductive surfaces of these sensor probes mostly need electrical insulating films coated on them, but it has been difficult to coat a high-quality and strong insulating film on a micro-acute probe such as a micropipette, especially on its tip. Therefore, we have employed diamond-like carbon (DLC) as an insulating film and developed a DLC coating method based on plasma chemical vapor deposition method. In the deposition apparatus, the cathode is the micropipette itself and the anode is a mesh cylinder with a central focus on the micropipette. In order to prevent the growing films from transformation due to high temperature at the tip, the voltage between the pair of electrodes is impressed intermittently. Raman spectrum and electrical resistivity measured here indicate that the deposited film is DLC and it can be worked well as an insulating film. The DLC coating method could be useful in micro-probe fabrications.**

Keywords—**Diamond-like Carbon (DLC), micropipette, plasma chemical vapor deposition, insulating film**

I. INTRODUCTION

Glass micropipettes are widely used in cell engineering and artificial insemination. They have micro-diameter hollow tips for injecting DNA fragments, fluorescent substances, or specific proteins into a cell [1]. They can also be used to measure electric potential at cell membrane as patch electrodes [2]. Furthermore, improving the micropipettes using material deposition and microfabrication techniques, micro-sensor probes measuring electric potential, pH, and chemical substance concentrations have been created [3-7].

Most of these sensor probes need to be coated with electrical insulating films on their conductive surfaces. For this purpose, silicon dioxide (SiO_2) and insulating organic polymers (e.g., polyimide and parylene), which are commonly used in micro-electrical device fabrications, may be available. These insulating materials can be easily coated on some flat substrates and smooth curved plates, but it is difficult to apply them to some acute wires and tubes. For the micropipette surface, SiO_2 films with homogeneous thickness and quality were hardly obtained neither with SiO_2 sputtering nor thermal oxidation of sputtered silicon in our experiments. In addition, the cracks of SiO_2 often appeared in the vicinity of the tip. On the other hand, the organic polymer has a disadvantage for being filled in the tip holes of the micropipettes after the deposition. Therefore, we have employed hydrogenated amorphous carbon, also generally called diamond-like carbon (DLC), as an electrical insulating film for the micropipette.

DLC has been much studied since the early 1970s due to its remarkable properties such as high electrical resistivity, very high hardness, low coefficient of friction, high thermal conductivity, chemical inertness, high optical (especially infrared) transparency, and biocompatibility [8-10]. Therefore, DLC coatings are lately used for various products in electronics, optics, and mechanics. With the recent evolution of micro-electro mechanical systems, DLC has also received more scientific interest for the applications in this field [11]. Using the interesting properties of DLC, novel micro-sensors are expected to be developed.

There are several methods to produce DLC films [8, 9]: plasma chemical vapor deposition (PCVD), ionized evaporation, arc ion plating, unbalanced magnetron sputtering, and others. However, all methods cannot achieve high quality DLC films on the micropipette surfaces. Among these, PCVD has several significant advantages for our purpose: low temperature deposition, little restriction on sample geometry, and simplicity of apparatus. We have thus employed PCVD, and modified its electrode configurations and voltage regulation method for DLC coating on the micropipettes. Consequently, the cathode has been the micropipette itself and the anode has been a mesh cylinder encompassing the cathode. Chemical structure and electric resistivity of the DLC films produced here were investigated with Raman spectroscopy and thin film resistance meter, respectively. Raman spectrum, especially peak positions, of a DLC film indicates its content ratio of sp^3 and sp^2 [9]. The measurement results show that the deposited films are DLC with high resistivity and thus they can be worked as insulating films.

II. METHOD OF DLC COATING

A. Preparation of Metal-Coated Glass Micropipettes

Glass micropipettes were produced by thermally tapering one ends of the pilex glass tubes with 1-mm-external diameter and 0.5-mm-internal diameter (G-1, Narishige, Japan), fully cleaned with acetone and alcohol in

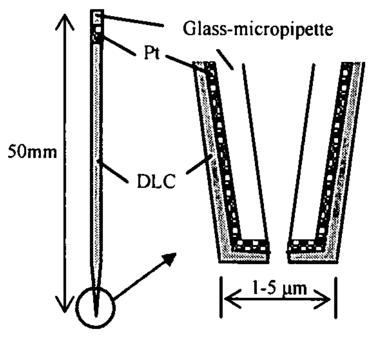

Fig. 1. DLC-metal-coated micropipette

advance, using the heating pipette puller (PB-7, Narishige, Japan). The diameter and the taper form can be changed with the pipette puller condition. We prepared 50-mm-long and 1 to 5-µm-tip diameter glass micropipettes as DLC coating targets, as shown in Figure 1.

In order to coat DLC on the micropipette with our deposition system described in the next section, the micropipette itself must be worked as a cathode, that is, an electrical conductor. Also, having conducting property derives a micropipette-electrode measuring electric potential, pH, or chemical substance concentration. Therefore, a metal film is needed on the glass micropipette. Here, titanium (Ti) and platinum (Pt) were deposited sequentially in the thickness of approximately 20 nm and 100 nm, respectively, on the glass micropipette outer surfaces using spattering method (Fig. 1). The Ti deposition is for good adhesion between Pt and glass. Subsequently they were annealed in 500 K for 120 min for better adhesion. The above metal-coated glass micropipette will be called as a '*probe*' hereafter.

B. DLC Coating Apparatus

We have deposited DLC on the probes with a modified PCVD system shown in Figure 2. In this system, methane (CH_4) gas is supplied into the main vacuum chamber in the flow rate of 10.0 sccm (standard cubic centimeter per minute), and the pressure is maintained at 25 Pa. The cathode is the probe itself, which is connected to the lower stainless steel tube cathode from the radio frequency (rf) voltage generator. The anode (grounded) is the mesh (#20, grid interval of 1 mm) cylindrical stainless steel with a central focus on the cathode. The rf impressed voltage generates the glow discharge and the dc voltage between a pair of the electrodes by self-bias effect [12]. The Teflon board can inhibit the discharge from the cathode under the probe. Therefore, the radial plasma of CH_4 can be formed only within the cylinder hollow anode, encompassing the micropipette cathode. The mesh form makes it possible to supply the gas from the outside to the inside of the cylinder hollow enough to hold the plasma. The cylinder diameter was optimized to achieve the homogeneity of the plasma, from the luminescent intensity observation, and to form the sheath around the cathode.

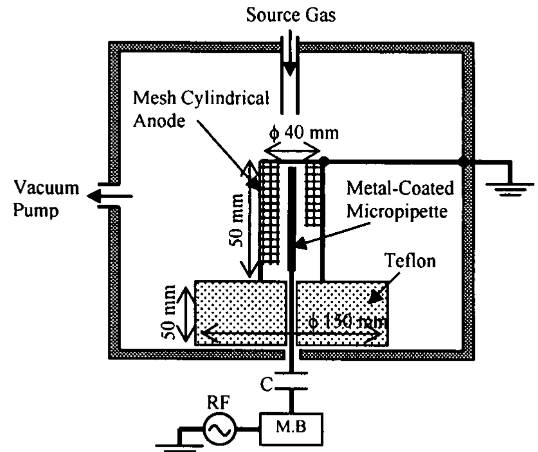

Fig. 2. Deposition apparatus

Fig. 3. Scanning electron micrograph of the tip

C. Voltage Control

To start glow discharge, the dc voltage between the pair of electrodes needs to be increased until dielectric breakdown of CH_4 gas. This threshold voltage is approximately -400 V. Subsequently, decreasing the voltage rapidly to a specified voltage, it is maintained for a specified duration. Initially, we set the dc voltage of -120 V, minimum voltage for keeping the glow discharge. Even in such a low voltage deposition for a few minutes, however, good deposition on the tip area was not achieved: transformations and/or detachments of the DLC films were seen. At the same time, the tips of the probes often bent slightly, which indicates that the tip temperatures became high enough to soften the pilex glass during deposition. When the temperature is too high, it is known that transformations proceed by dehydrogenation and graphitization, that is, sp^3 is converted into sp^2 bonded carbon.

The temperature rise can be attributed to the concentration of the ion collision with the growing film on

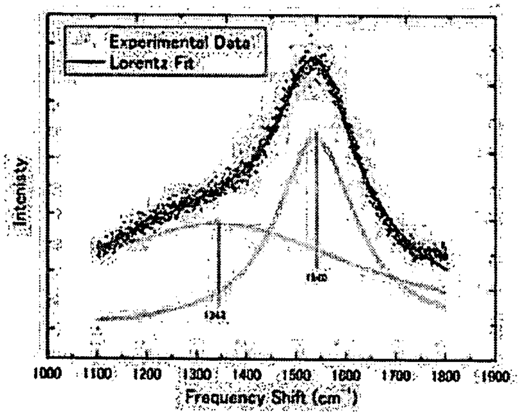

Fig. 4. Raman spectrum of a deposited DLC film

the probe tip where the electric flux lines are in the highest density [13]. Additionally, since the thermal capacity of the tip is very small and the thermal diffusion rate is much less than that of a plane substrate, the high temperature region can be easily generated.

In order to prevent the DLC film on the tip from the temperature rise, it must be useful to shorten the deposition duration. We have thus iterated the short-time deposition and the cooling interval. Consequently, we have executed 10-s-deposition and 60-s-interval, automatically regulating the rf voltage generator output. It is necessary for thick film deposition to increase the total number of the cycles. Using this voltage control method, DLC films can be produce without detachment throughout the surface, including the tip. Figure 3 shows a scanning electron microscope image of the tip.

III. Structural and Electrical Characterization

A. Raman Spectrum

Formation of DLC is still conjectural, but it is noted that the property of DLC strongly depends on content of sp^2 and sp^3 bonds, which can be estimated with Raman spectra, especially peak positions [14]. The Raman spectra of the DLC films deposited here were obtained using the Raman spectroscopy developed by Abe [15] and analyzed. Figure 4 shows that the measured spectrum contains two peaks, generally called 'G-peak' (1540 cm^{-1}) and 'D-peak' (1343 cm^{-1}). This spectrum is similar to a typical one reported in the past.

B. Electrical Resistivity

Electrical resistivity of the DLC film on the probe was measured using a thin film resistance measurement

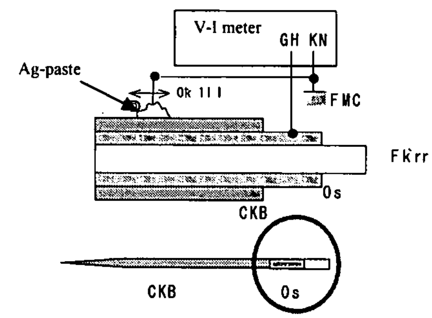

Fig. 5. Illustration of measurement system of the DLC film resistivity

apparatus with a voltage-current meter, as shown in Figure 5. In order to calculate resistivity, thickness of the DLC film and area of the argent-paste are needed. The thickness was measured at the boundary step of the DLC film in the end opposite of the tip using a surface profilometer (Dektak^{TM} 3030ST, Veeco). In the total deposition time of 360 s (10 s times 36 cycles), the thickness was approximately 40 nm. The average resistivity was 10^{14} Ωm. This value is within the reported resistivity of a DLC film: 10^7 to 10^{14} Ωm [9]. The DLC films produced here fully work as electrical insulating films.

IV. Discussion

Using a PCVD apparatus with a mesh cylindrical anode and intermittent voltage impressions, we were able to successfully coat DLC films on the probes. This deposition system is very simple. Modifying an electrode configuration of a conventional two-electrode-type PCVD apparatus, the system introduced here can be set up easily and inexpensively. The combination of the mesh cylinder as an anode and the probe as a cathode can yield a homogeneous and steady plasma circumferentially and longitudinally for the probe.

In addition to the electrode configuration, the voltage impression method is important for our deposition system. Transformation of a DLC film due to high temperature is the same problem for some acute wires and thin tubes. Although this method cannot be applied to all kinds of microprobes, it could be effective for some probes whose tip sizes are larger than the micropipette at least. The longitudinal thermal conduction in the glass and the thermal radiation to the environment are dominant factors for the heat transfer in this case. If there is a good method quenching the tip, it may inhibit the DLC transformation instead of the voltage control.

Since DLC has not only high resistivity but also other interesting properties, especially high hardness and high optical transparency, they may enhance the possibilities of

the microprobes. For example, the high hardness contributes to strengthening the tip, and thus the micropipette might have high penetration ability. Also, it might be able to inject a certain liquid with high pressure. Furthermore, a lot of doping methods have been studied to give DLC novel property. The DLC coating method would be helpful to the future development of microprobes.

When producing an electrode probe for a microscale potential measurement, it is necessary to eliminate a part of the insulating film on the probe, mostly on its tip, to expose the metal surface. Chemical, mechanical, and electrical ablation techniques [16] have been considered, but it is difficult to strip a specified micro-area of the insulating films, especially the DLC films due to its chemical inertness and hardness. In the present microfabrication techniques, focused ion beam etching could be most effective [7]. We have also used it for the glass micropipette tip etching [17] and confirmed its validity for the DLC film. In this method, a 1-μm-region from the end is cut off, and theoretically the ring-shaped metal surface would appear on the end face.

V. Conclusion

We have developed a method for coating DLC as an insulating film on a metal-coated micropipette which is widely used in cell engineering. In a simple plasma chemical vapor deposition system, electrode configuration and voltage regulation method have been modified for our purpose. The cathode is the probe itself and the anode is a mesh cylinder with a central focus on the probe. In order to prevent the growing films at the tip from transformation due to high temperature, the voltage between the pair of electrodes is impressed intermittently. Raman spectrum and electric resistivity measured here indicated that the deposited film was DLC and it can be worked as an insulating film. The DLC coating method described here could be applied not only to micropipettes but also to some micro-acute wires and tubes. Therefore, the DLC coating would be useful in various probe fabrications.

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